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Morphology and Taxonomy of *Cocconeis subtilissima* Meister (*Bacillariophyceae*) and Two Closely Related Taxa from the Coastal Waters of Japan

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Morphological and taxonomical observations of a marine attached diatom *Cocconeis subtilissima* Meister have been made. The external and internal structure of raphid and araphid valves and the complete cingulum structure are described using a light microscope and scanning and transmission electron microscopes. Comparison of *C. subtilissima* and two closely related taxa, *C. pellucida* Grunow and *C. pseudomarginata* Gregory var. *intermedia* Grunow, shows important ultrastructural differences. The arrangement of foramen along the axial area and the number of their rows in the araphid valve are most distinctive characteristics. *C. subtilissima* has smoothly bowed two to three rows. The other two taxa have a smoothly bowed one and slightly undulate five to six ones, respectively.

Key words: *Cocconeis subtilissima*, Japan, marine diatom, morphology, taxonomy.

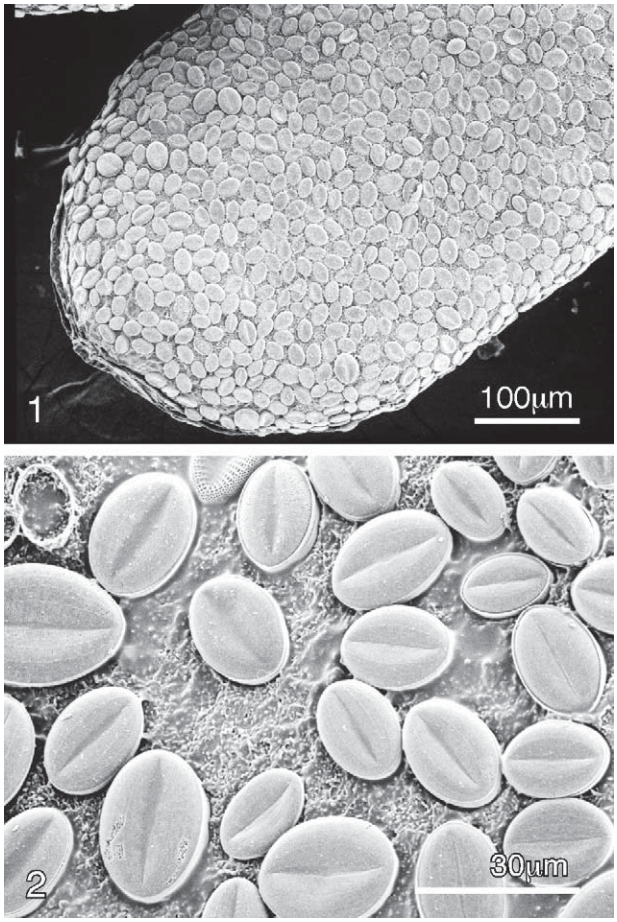
Cocconeis subtilissima Meister (1934) was originally described from Nagasaki Pref., Japan, and has hitherto been known from the coastal waters of Japan with a brief description (Nagumo and Tanaka 1990, Suzuki et al. 2000, Nagumo et al. 2000, Suzuki and Nagumo 2003, 2004). Recently De Stefano and Romero (2005) classified this species into the new section "*Alveolatae*" of the genus *Cocconeis* based on the araphid valve (ARV) with chambered alveoli. However, detailed structures, such as areolae, hymen and cingulum, on *C. subtilissima* were not described in detail in these previous papers. This is the first report on the important diagnostic criteria of this species, with emphasis on the structure of areolae and cingulum, and continuing on the following papers dealing with the systematic relationships of *Cocconeis*.

Materials and Methods

The taxa examined in the present investigation were collected from the following localities: JAPAN: Kyushu, Nagasaki Pref., Nagasaki Peninsula, Nomozaki (32°34′N, 129°47′E), 31 March 2000, epiphytic on *Corallina pilulifera* Postels & Ruprecht (*Corallinaceae*, *Rhodophyceae*), H. Suzuki s.n. (MTUF-AL-HS 0195); Honshu, Tokyo, the Izu Islands, Miyake-jima Island, Hijikata (34°04′N, 139°32′E), 30 May 1998, epiphytic on *Chlorodesmis caespitosa* J. Agardh (*Codiaceae*, *Chlorophyceae*), A. Kobayashi s.n. (MTUF-AL-HS 0007); Shikine Island, Jinata (34°18′N, 139°13′E), 09 May 1998,

epiphytic on *Caulerpa okamurae* Weber van Bosse f. *okamurae* (*Caulerpaceae*, *Chlorophyceae*), H. Suzuki s.n. (MTUF-AL-HS 0005); Kanagawa Pref., Ashigarashimo-gun, Manazuru-machi, Manazuru-misaki, Mitsuishi (35°08′N, 139°09′E), 29 April 2002, epiphytic on *Corallina pilulifera*, H. Suzuki s.n. (MTUF-AL-HS 0389); Miura-gun, Hayama-machi, Shimoyamaguchi, Koiso (35°15′N, 139°34′E), 25 September 2002, epiphytic on *Corallina pilulifera*, H. Suzuki s.n. (MTUF-AL-HS 0405).

All materials were treated using a bleaching method (Nagumo and Kobayasi 1990, Nagumo 1995, Osada and Nagumo 2001). Light and electron microscopy techniques were essentially the same as those previously used (cf. Suzuki et al. 2001a, 2001b, 2001c, 2001d). Specimens were examined using Hitachi S-4000 and Hitachi S-5000 SEMs, and a JEOL-200EX TEM. Striae densities were measured along the axial area near the center of a valve. The terminology is followed by Anonymous (1975), Ross et al. (1979) and Round et al. (1990) using additional terms from Holmes et al. (1982) and Kobayasi et al. (2006).



Figs. 1, 2. Scanning electron micrographs of *Cocconeis subtilissima*, epiphytic on *Corallina pilulifera*.

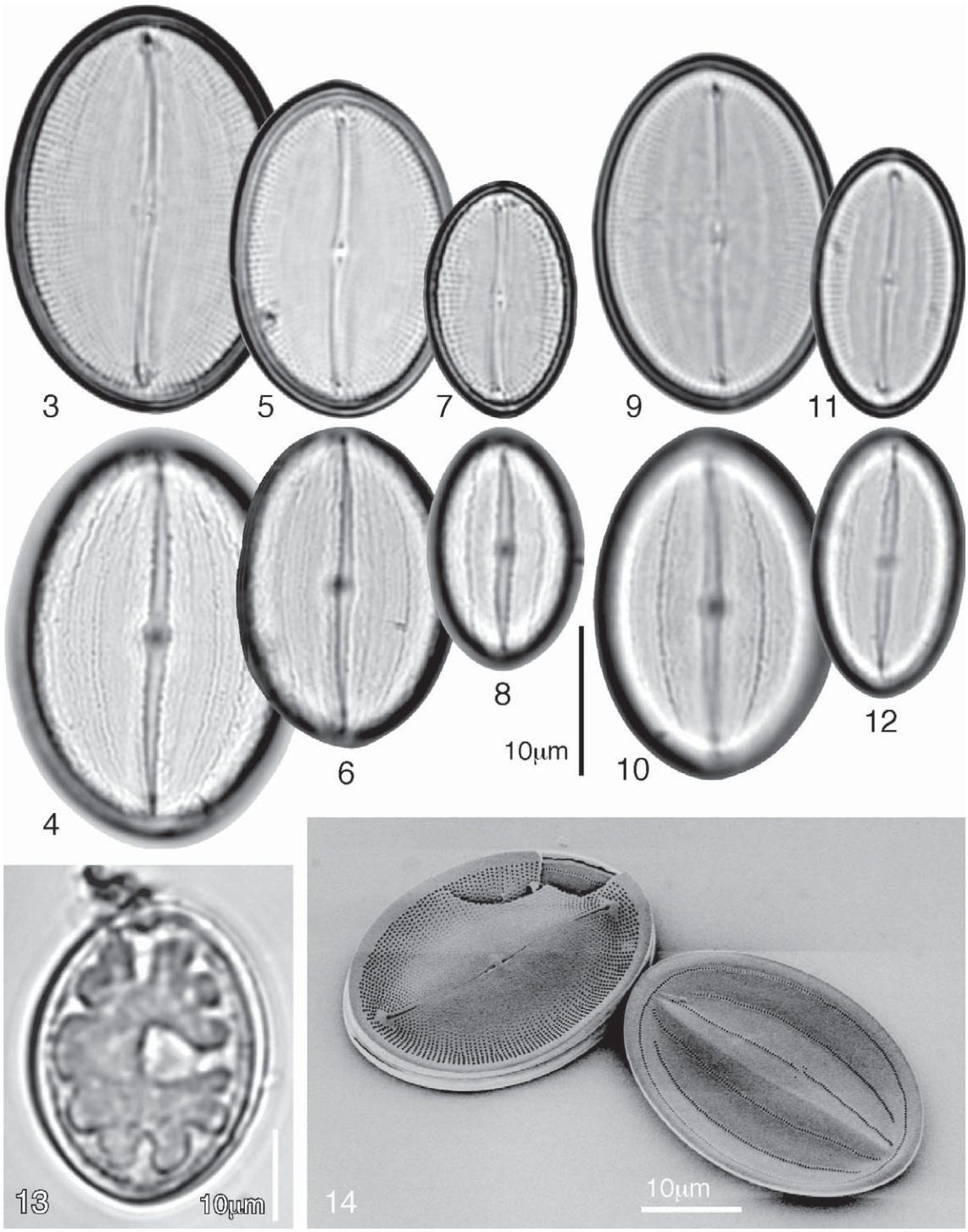
Results and Discussion

Cocconeis subtilissima Meister in Ber. Schweiz. Bot. Ges. **44**: 99, pl. 7, figs. 61, 62 (1934); Nagumo & Ji. Tanaka in Mem. Natn. Sci. Mus., Tokyo (23): 16, 20, figs. 11a, b (1990); Hide. Suzuki & al. in Bull. Aoyama Gakuin Senior High School **23**: 19, pl. 4, figs. 1–4 (2000); Nagumo & al. in Bull. Nippon Dental Univ., General Education **29**: 218, pl. 21, figs. 95a, b (2000); Hide. Suzuki & Nagumo in Bull. Nippon Dental Univ., General Education **32**: 110, pl. 1, figs. 1, 2 (2003), **33**: 62, pl. 1, figs. 1–4 (2004); De Stefano & Romero in Bibl. Diatomologica **52**: 38, 39, pl. 32, figs. 1–9, pl. 33, figs 1–11 (2005).

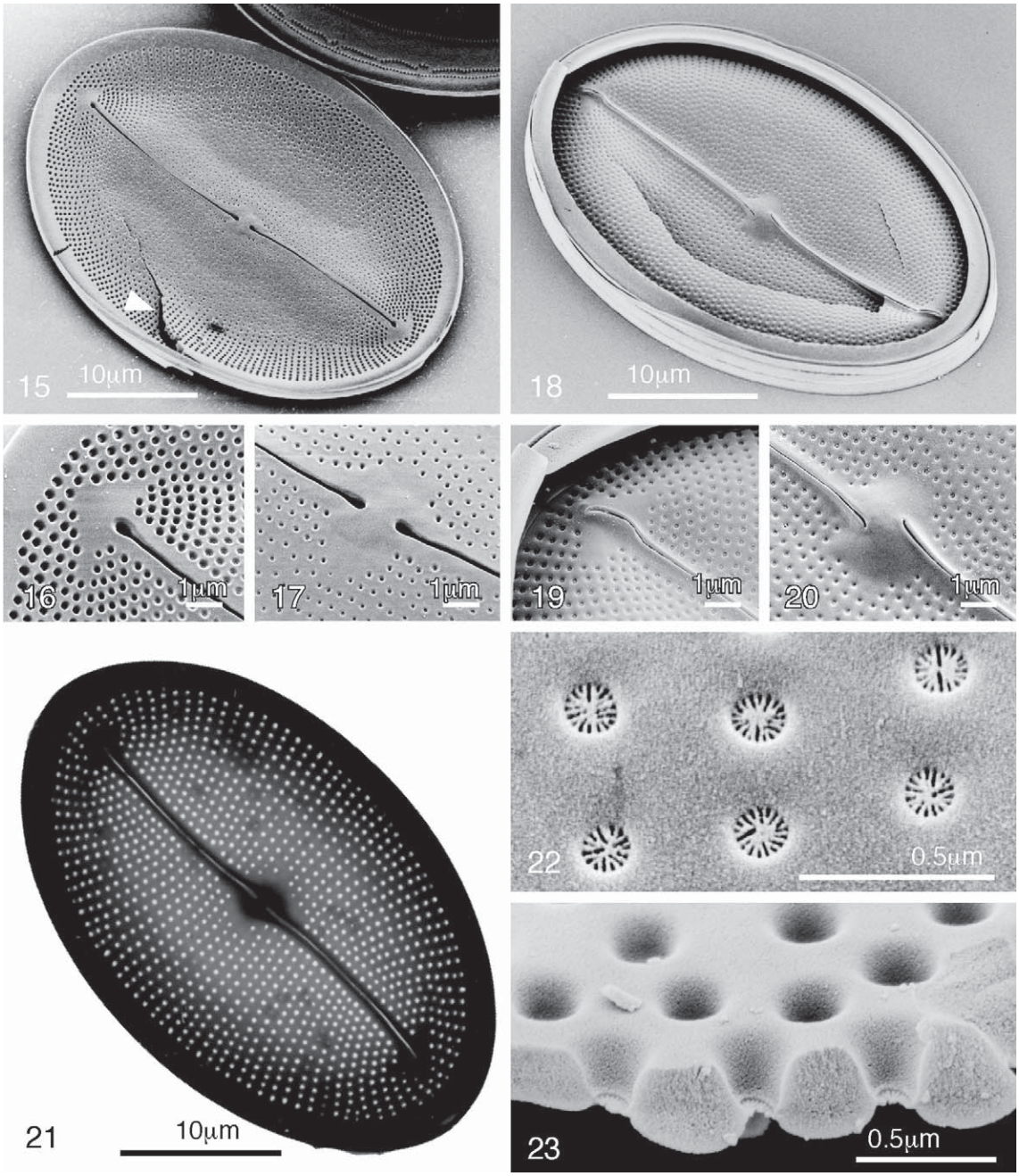
In the collections examined here, *Cocconeis subtilissima* is epiphytic on the raphid valve (RV) closely appressed to the surface of a red alga *Corallina pilulifera* in company with *Cocconeis scutellum* Ehrenberg and other diatom taxa (Figs. 1, 2).

Frustules have elliptic valves, which are very dissimilar to each other, varying in outline from narrow and broadly elliptical (Figs. 3–12, 14), 14.0–45.0 μm long, 8.5–39.0 μm wide. Striae densities at the center of valves are 26–28 in 10 μm on the RV and 28–30 in 10 μm on the ARV. The single plastid is flat and C-shaped, and is either simple or elaborately lobed in outline as in some other *Cocconeis* taxa (Fig. 13; cf. Suzuki et al. 2001b, 2001c, 2001d).

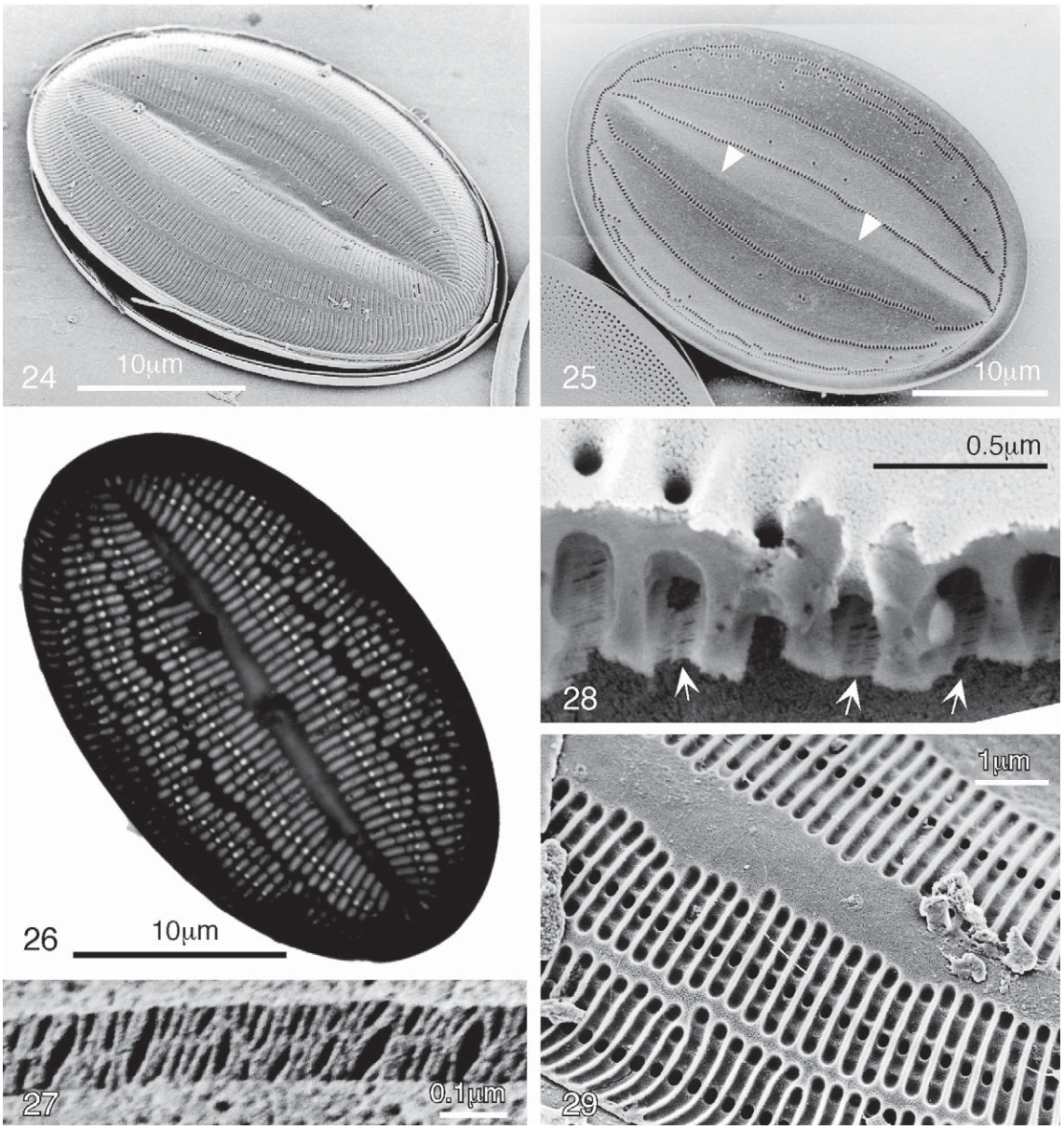
The RV is concave and not elevated in the axial area (Fig. 15). The raphe appears as a simple, slightly sigmoid slit (Figs. 15, 21) as



Figs. 3–14. Light micrographs (3–13) and scanning electron micrograph (14) of *Cocconeis subtilissima*. 3–12. Raphid valves (3, 5, 7, 9, 11) and araphid valves (4, 6, 8, 10, 12) of the same frustules. Note that the frustules are variable in shape and length. 3–8. Nomozaki (MTUF-AL-HS 0195). 9–12. Miyake-jima Island (MTUF-AL-HS 0007). 13. Living cell showing a C-shaped and elaborately lobed plastid. 14. Raphid valve (left) and araphid valve of the same frustule treated using the bleaching method.



Figs. 15–23. Scanning electron micrographs (15–20, 22, 23) and transmission electron micrograph (21) of raphid valves of *Cocconeis subtilissima*. 15. External view of raphid valve of the same frustule as araphid valve of Fig. 25. 16. External view of raphid valve showing the distal raphe end and the terminal area. 17. External view of raphid valve showing the proximal raphe ends and the central area. 18. Internal view of raphid valve with a valvocopula and two bands of raphid valve and a valvocopula of araphid valve. 19. Internal view of raphid valve showing the lightly deflected helictoglossae. 20. Internal view of raphid valve showing the proximal raphe ends and the central area. 21. Raphid valve showing the sigmoid raphe and the uniseriate striae consisting of small and round areolae. 22. Hymens with linear perforations in a centric array. 23. Enlarged, the part marked with an arrowhead of Fig. 15. Note the pore occlusions located near the inner surface of a broken raphid valve.



Figs. 24–29. Scanning electron micrographs (24, 25, 27–29) and transmission electron micrograph (26) of araphid valves of *Cocconeis subtilissima*. 24. External view of araphid valve. 25. Internal view of araphid valve of the same frustule as raphid valve of Fig. 15. Arrowheads indicate a vestigial raphe on the axial area. 26. Araphid valve showing the alveolate striae and the foramina. 27. Hymens with perforations in a parallel array. 28. Alveoli occluded by hymens (arrows) located near the outer surface. 29. External face of immature araphid valve, showing alveoli, each with one foramen.

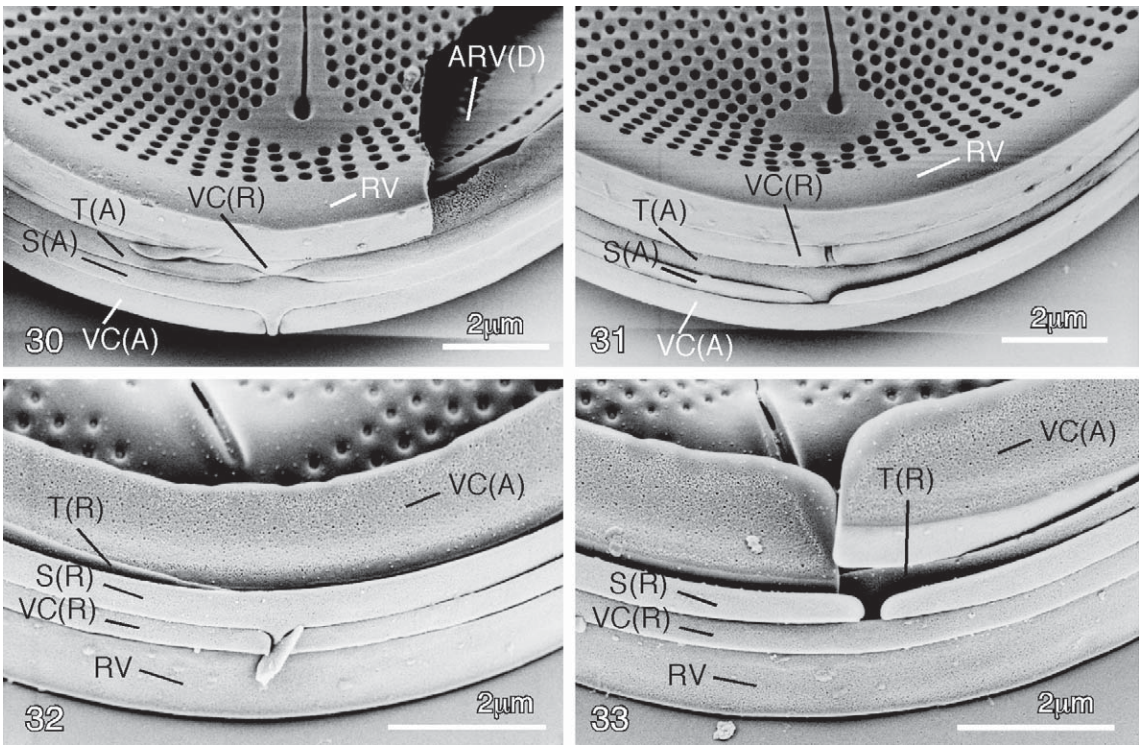
in *C. pellucida* Grunow (Kobayasi and Nagumo 1985, De Stefano and Romero 2005) and *C. pseudomarginata* Gregory var. *intermedia* Grunow (Suzuki et al. 2001d, De Stefano and Romero 2005) that is not more curved than of *C. heteroidea* Hantzsch (Suzuki et al. 2001a, De Stefano and Romero 2005). The inner raphe fissures lie in a very narrow and raised, axial area (Fig. 18). The proximal raphe ends are coaxial and somewhat dilated externally (Fig. 17), but undilated and deflected in the opposite direc-

tions internally (Fig. 20), just as in most *Cocconeis* taxa investigated so far (Kobayasi and Nagumo 1985, Round et al. 1990, Romero 1996, Romero and Rivera 1996, Suzuki et al. 2001a, 2001b, 2001c, 2001d, Sar et al. 2003, De Stefano and Romero 2005). The distal raphe ends are dilated externally (Fig. 16), but terminate in short, narrow and small helictoglossae internally, which are turned in the opposite directions (Figs. 18, 19). The central area is round and small, appearing flat externally and thickened internally (Figs. 17, 20). Each terminal area forms an arrow-head shaped hyaline area that is visible externally and internally (Figs. 16, 19), as observed in *C. pellucida* (Kobayasi and Nagumo 1985, De Stefano and Romero 2005), *C. heteroidea* (Suzuki et al. 2001a, De Stefano and Romero 2005) and *C. pseudomarginata* var. *intermedia* (Suzuki et al. 2001d, De Stefano and Romero 2005). The striae consisting of small and round areolae are uniseriate and radiate (Fig. 21). Each areola is circular and occluded by a hymen with perforations arranged in a centric array (Fig. 22; cf. Mann 1981). These hymens are located near the internal surface of the valve (Fig. 23). A broad marginal hyaline ring is visible. The submarginal external area and internal thickening, which are recognized in *C. scutellum* Ehrenberg (Holmes et al. 1982, Lange-Bertalot and Krammer 1989, Romero 1996), are absent (Figs. 15, 21).

The ARV is slightly convex (Fig. 24), being complementary to the RV. The ARV is thicker than the RV, which is one of the important morphogenetic features in this genus (Round et al. 1990, De Stefano and Romero 2005). The axial area is a narrow, slightly sigmoid lanceolate depression on the outer surface (Figs. 24, 26), which is always prominent. In some valves, a vestigial raphe is observed (Fig. 25, arrowheads). A longitudinal hyline area, running parallel to the axial area, bisects the alveolate striae (Figs. 24, 26). Internally, each alveolus opens by means of a circular foramen (Fig. 25). One set of foramen forms an ellipse along the valve margin, while the others form three or four parallel to the hyaline areas (Fig. 25). Each alveolus is occluded by a hymen located near the outer surface (Fig. 28, arrows). Hymen perforations are arranged in a parallel array (Fig. 27; cf. Mann 1981). Observations of the external face of an immature ARV illustrate the structure of the internal perforations of alveoli (Fig. 29).

The mature cingulum of both valves consist of at least three girdle bands; a valvocopula and two bands (Figs. 30–33). The valvocopula of each valve is open at one pole of the cell, and not furnished with fimbriae (Figs. 34–37). The second and the third bands are narrower and thinner than the valvocopula. The second band is an open band with a ligula (Figs. 38, 39). The third band is also open and has a small ligula (Figs. 40, 41). Each band adjacent to the valvocopula is open reciprocally at the opposite poles (Figs. 30–33). A similar structure has been observed in the valvocopula of *C. heteroidea* (Suzuki et al. 2001a), *C. convexa* Giffen (Suzuki et al. 2001c), *C. pellucida* (Kobayasi and Nagumo 1985, De Stefano and Romero 2005) and *C. pseudomarginata* var. *intermedia* (Suzuki et al. 2001d).

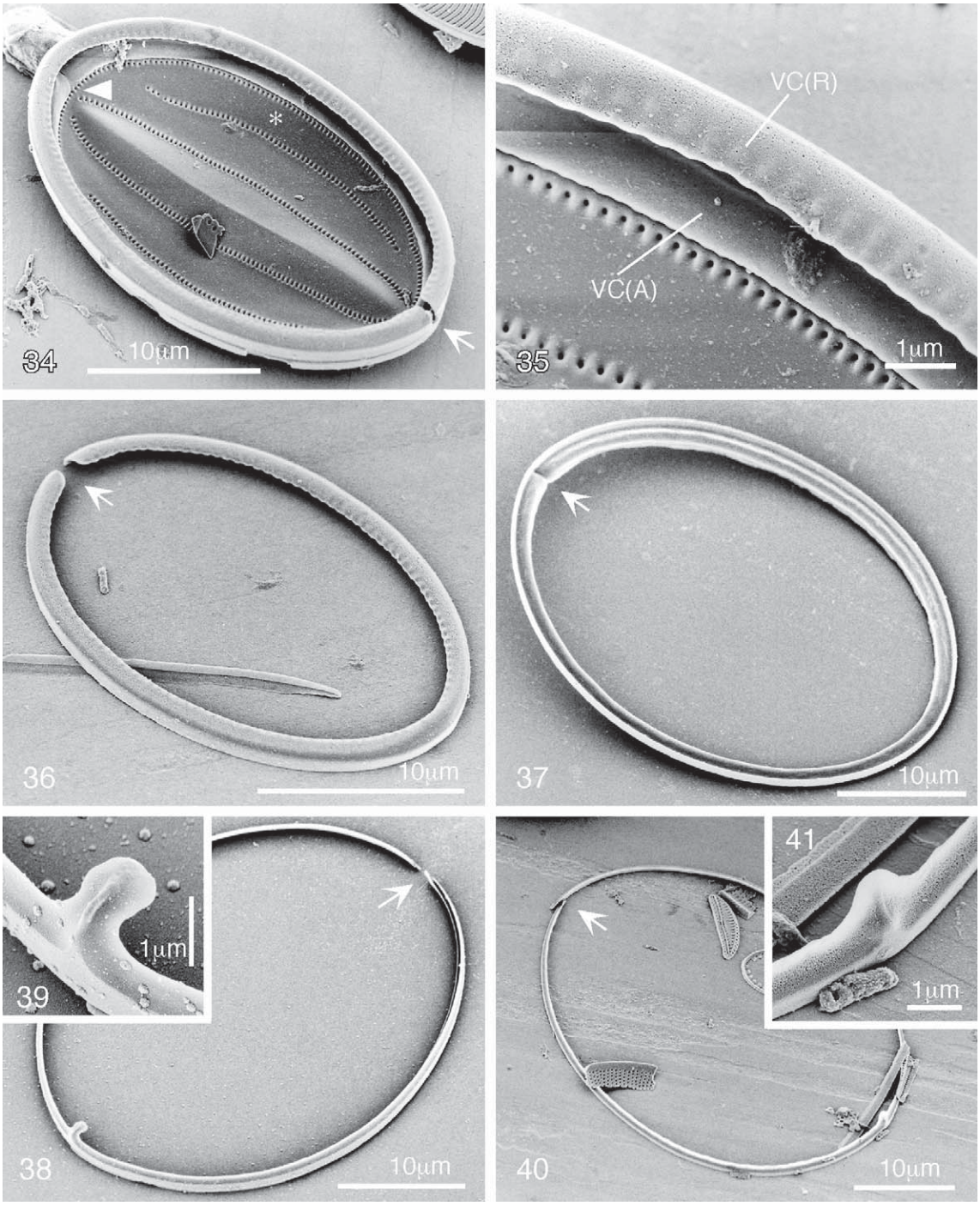
The descriptions and illustrations given by us here and given by Kobayasi and Nagumo (1985), Suzuki et al. (2001d), and De Stefano and Romero (2005) show that *C. subtilissima*, *C. pellucida*, and *C. pseudomarginata* var. *intermedia* are closely related species with each other and may be distantly connected to the type species of *Cocconeis*, *C. scutellum* (Lange-Bertalot and Krammer 1989, Romero 1996). For instance, all of these have common characteristics as follows; (i) a sigmoid raphe, the absence of a submarginal hyaline, uniseriate striae consisting of round areolae, and hymen with perforations arranged in a centric array in the



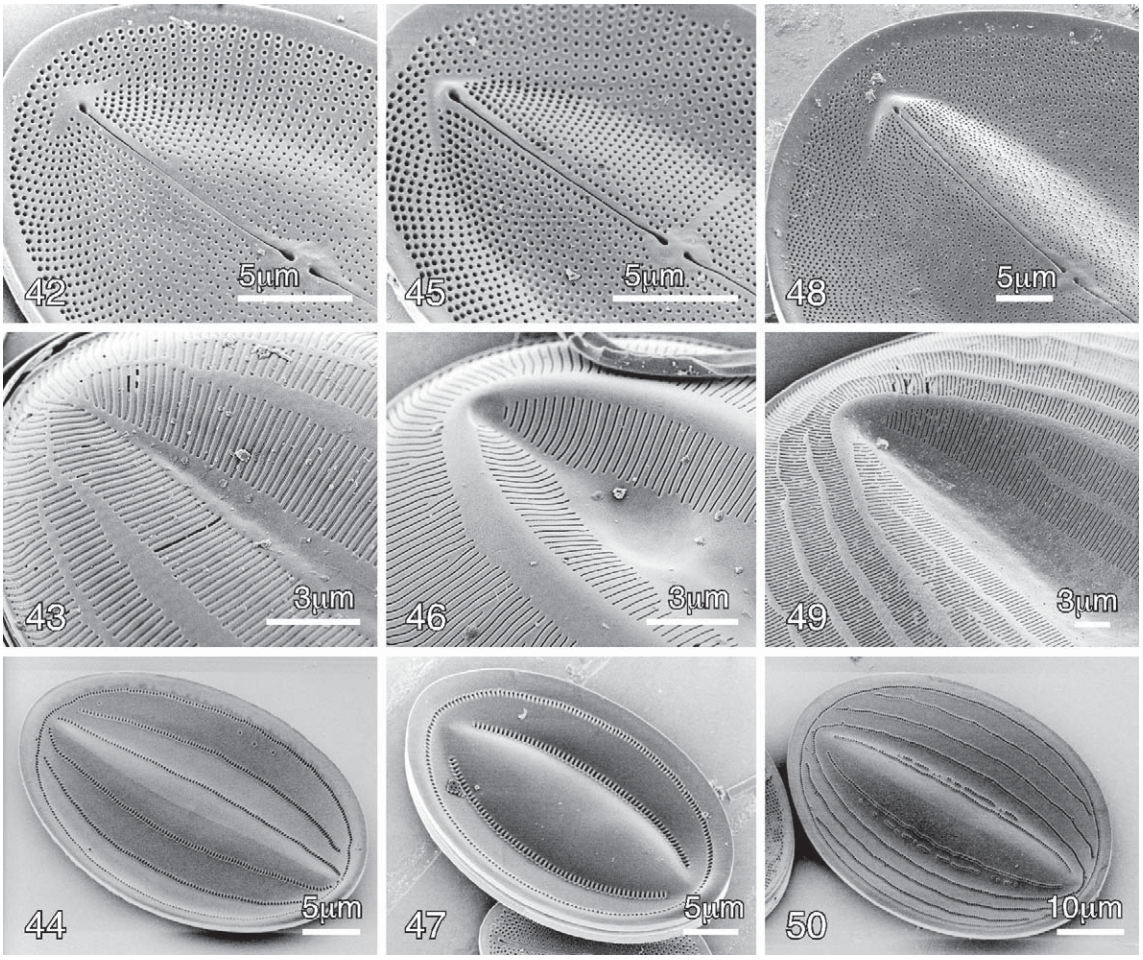
Figs. 30–33. Scanning electron micrographs of cingula of *Cocconeis subtilissima*. 30, 31. Both distal ends of the raphid valve of the same individual as Fig. 14. 32, 33. Both distal ends of the raphid valve of the same individual as Fig. 18. ARV(D). Daughter araphid valve. VC(A), S(A) and T(A). Valvocopula, the second band and the third band of araphid valve, respectively. VC(R), S(R) and T(R). Valvocopula, the second band and the third band of raphid valve, respectively.

RV; (ii) the alveolate areolae and the hymen with linear perforations in parallel array in the ARV, and (iii) the open smooth valvocopulae of both valves, which are stable characteristics. Thus, if these species were abundant as the epiphytic diatoms in the same sample (e.g., Suzuki and Nagumo 2003), it was difficult to accurately distinguish them. In this study, we are able to demonstrate that there are some marked differences between them in the valve structure. Specifically, the valve surface observed by SEM has been found to be useful for distinguishing and characterizing the three *Cocconeis* taxa. These differences are recognizable even under LM, and the accurate identification could be made by a combination of several characteristics. Comparisons between these taxa are summarized in Figs. 42–50 and Table 1. The arrangement of foramen along the axial area and the number of their rows in the ARV are most distinctive characteristics. In *C. subtilissima*, *C. pseudomarginata* var. *intermedia*, and *C. pellucida*, the ARVs have two to three smoothly bowed rows (Figs. 43, 44), a smoothly bowed one (Figs. 46, 44), and five to six slightly undulate ones (Figs. 49, 50) respectively. On a hollow in the axial area in the ARV, *C. subtilissima* is narrow and shallow compared with the other two. In addition, *C. pellucida* is characterized by the larger valve size and the lower density of striae in RV (Figs. 42, 45, 48).

We are grateful to Dr Atsushi Kobayashi, Keika Boy's Junior & Senior High School, for providing specimen treated herein. This



Figs. 34–41. Scanning electron micrographs of cingula of *Cocconeis subtilissima*. 34. Internal view of araphid valve with valvocopula attached, and valvocopula of raphid valve. Arrowhead indicates an open part of valvocopula of araphid valve, and an arrow indicates an open part of valvocopula of raphid valve. 35. Enlarged the part marked with an asterisk in Fig. 34. VC(A). Valvocopula of araphid valve. VC(R). Valvocopula of raphid valve. 36. Valvocopula of raphid valve. Arrow indicates its opening at one apex. 37. Valvocopula of araphid valve. Arrow indicates its opening at one apex. 38, 39. The second band of araphid valve with a ligula (39). Arrow indicates its opening at one apex. 40, 41. The third band of araphid valve with a ligula (41). Arrow indicates its opening at one apex.



Figs. 42–50. Scanning electron micrographs for comparison of the main morphological characteristics among *Cocconeis subtilissima* (42–44), *C. pseudomarginata* var. *intermedia* (45–47), and *C. pellucida* (48–50) from Japanese coastal waters. 42, 45, 48. Raphid valves, showing terminal and central areas, external views. 43, 46, 49. Araphid valves, showing terminal and central areas, external views. 44, 47, 50. Whole araphid valves, internal views.

Table 1. Comparison of *Cocconeis subtilissima* with *C. pseudomarginata* var. *intermedia* and *C. pellucida* in morphological attributes

Characteristics	*C. subtilissima*[c, d]	*C. pseudomarginata* var. *intermed*[b, d]	*C. pellucida*[a, d]
Apical axis (μm)	14.0–45.0	18.5–59.0	45.0–61.0
Transapical axis (μm)	8.5–39.0	12.0–47.0	32.0–46.0
Raphid valve			
Density of striae in 10 μm	26–28	26–28	14–20
Araphid valve			
Density of striae in 10 μm	28–30	26–32	32–36
Foramina			
Arrangement	smooth	smooth	slightly undulate
Number of row	2–3	1	5–6
Hollow of axial area	narrow and shallow	broad and deep	broad and deep

[a]Kobayasi and Nagumo (1985), [b]Suzuki et al. (2001d), [c]De Stefano and Romero (2005), [d]the present study.

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References

Anonymous. 1975. Proposals for a standardization of diatom terminology and diagnoses. Nova Hedwigia, Beih. **53**: 323–354.

De Stefano M. and Romero O. 2005. A survey of alveolate species of the diatom genus *Cocconeis* (Ehr.) with remarks on the new section *Alveolatae*. Bibl. Diatomologica **52**: 1–133.

Holmes R. W., Crawford R. M. and Round F. E. 1982. Variability in the structure of the genus *Cocconeis* Ehr. (*Bacillariophyta*) with special reference to the cingulum. Phycologia **21**: 370–381.

Kobayasi H. and Nagumo T. 1985. Observations on the valve structure of marine species of the diatom genus *Cocconeis* Ehr. Hydrobiologia **127**: 97–103.

Kobayasi H., Idei M., Mayama S., Nagumo T. and Osada K. 2006. H. Kobayasi's Atlas of Japanese Diatoms Based on Electron Microscopy 1. 533 pp. Uchida Rokakuho, Tokyo.

Lange-Bertalot H. and Krammer K. 1989. *Achnanthes*: eine Monographie der Gattung mit Definition der Gattung *Cocconeis* und Nachträgen zu den *Naviculaceae*. Bibl. Diatomologica **18**: 1–393.

Mann D. G. 1981. Sieves and flaps: Siliceous minutiae in the pores of raphid diatoms. *In*: Ross R. (ed.), Proceedings of the Sixth Symposium on Recent and Fossil Diatoms. pp. 279–300. Otto Koeltz, Koenigstein.

Meister F. 1934. Seltene und Kieselalgen. Ber. Schweiz. Bot. Ges. **44**: 87–108.

Nagumo T. 1995. Simple and safe cleaning methods for diatom samples. Diatom **10**: 88.

Nagumo T. and Kobayasi H. 1990. The bleaching method for gently loosening and cleaning a single diatom frustule. Diatom **5**: 45–50.

Nagumo T. and Tanaka J. 1990. Epiphytic diatoms on red algae in Tokunoshima Island of the Amami Islands. Mem. Natn. Sci. Mus., Tokyo (23): 15–21 (in Japanese with English summary).

Nagumo T., Kobayashi A., Hasegawa K., Doi, Y., Ishitsuna A., Osada K. and Tanaka J. 2000. Seaweeds, seagrasses and marine diatoms of Sado Island, Niigata Prefecture (Preliminary report). Bull. Nippon Dental Univ., General Education **29**: 183–222 (in Japanese with English summary).

Osada K. and Nagumo T. 2001. An introduction to diatom research. Bull. Nippon Dental Univ., General Education **30**: 131–142.

Romero O. E. 1996. Ultrastructure of four species of the diatom genus *Cocconeis* with the description of *C. pseudocostata* spec. nov. Nova Hedwigia **63**: 361–396.

Romero O. E. and Rivera P. 1996. Morphology and taxonomy of three varieties of *Cocconeis costata* and *C. pinnata* (*Bacillariophyceae*) with considerations of *Pleuroneis*. Diat. Res. **11**: 317–343.

Ross R., Cox E. J., Karayeva N. I., Mann D. G., Paddock T. B. B., Simonsen R. and Sims P. A. 1979. An amended terminology for the siliceous components of the diatom cell. Nova Hedwigia, Beih. **64**: 513–533.

Round F. E., Crawford R. M. and Mann D. G. 1990. The Diatoms. 747 pp. Cambridge University Press, Cambridge.

Sar E. A., Romero O. and Sunesen I. 2003. *Cocconeis* Ehrenberg and *Psammococconeis* Garcia (*Bacillariophyta*) from the Gulf of San Matías, Patagonia, Argentina. Diat. Res. **18**: 79–106.

Suzuki H. and Nagumo T. 2003. Epiphytic diatoms on a red alga *Corallina pilulifera* Postels et Ruprecht from Hayama Town, Kanagawa Prefecture, Japan. Bull. Nippon Dental Univ., General Education **32**: 109–118 (in Japanese with English summary).

Suzuki H. and Nagumo T. 2004. Epiphytic diatoms on a red alga *Asparagopsis taxiformis* (Delile) Trevisan from Hachijo Island, Izu Islands, Japan. Bull. Nippon Dental Univ., General Education **33**: 61–71 (in Japanese with English summary).

Suzuki H., Nagumo T. and Tanaka J. 2000. Epiphytic diatoms on a red alga *Ceramium tenerrimum* (Martens) Okamura from Kanagawa Pref., Japan. Bull. Aoyama Gakuin Senior High School **23**: 13–25 (in Japanese with English summary).

Suzuki H., Nagumo T. and Tanaka J. 2001a. Morphology of the marine epiphytic diatom *Cocconeis heteroidea* (*Bacillariophyceae*). Phycol. Res. **49**: 129–136.

Suzuki H., Nagumo T. and Tanaka J. 2001b. A new marine diatom *Cocconeis shikinensis* sp. nov. (*Bacillariophyceae*) from Japan. Phycol. Res. **49**: 137–144.

Suzuki H., Nagumo T. and Tanaka J. 2001c. Morphology of the marine epiphytic diatom *Cocconeis convexa* Giffen (*Bacillariophyceae*). Diatom **17**: 59–68.

Suzuki H., Nagumo T. and Tanaka J. 2001d. Morphology of the marine diatom *Cocconeis pseudomarginata* Gregory var. *intermed* Grunow. Diat. Res. **16**: 93–102.

鈴木秀和[a]，南雲　保[b]，田中次郎[a]：**海産付着珪藻 *Cocconeis subtilissima* Meister（珪藻綱）と近縁種の形態と分類**

本邦沿岸域から採集した海藻に着生していた *Cocconeis subtilissima* Meister について，光学顕微鏡，走査型および透過型電子顕微鏡による殻微細構造の観察結果を報告する．殻は楕円形．色素体はC字形．縦溝殻：殻は薄く少し凹状．縦溝はややS字状，外裂溝は中心側および極側でともに広がって終わる．内裂溝は盛り上がった狭い軸域にあり，中心側末端は先細りになり，殻縁部方向に曲がる。極側末端は蝸牛舌状で終わる．条線は1列の胞紋からなる．胞紋は小さな円形で，殻の内側に近い位置に薄皮が張る．薄皮は中心配列の穿孔をもつ．無縦溝殻：殻は厚く凸状に湾曲する．条線は2～3個の長胞胞紋からなる．長胞胞紋の外側の開口はスリット状で，内側の開口は円形の小孔をなす．殻内面の小孔列は，長軸に沿ったなめらかな曲線と殻縁部に沿った楕円形をなす．薄皮は殻の外側に近い位置に張り，平行配列の穿孔をもつ．半殻帯：両殻とも3枚の帯片をもつ．すべて片端開放型で鋸歯状突起をもたない．接殻帯片はもっとも幅が広く，第2および第3帯片は小舌を有する．形態的には，本種は *C. pseudomarginata* Gregory var. *intermedia* Grunow と *C. pellucida* Grunow に似ている．しかし，無縦溝殻の長胞胞紋の内側開口部の配列やその列の数が前者はなめらかな曲線で1列，後者はややジグザグ状で5～6列になる点で，本種と異なっている．

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